

# Performance of the Nzi and other traps for biting flies in North America

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## Abstract

The performance of Nzi traps for tabanids (*Tabanus similis* Macquart, *T. quinquevittatus* Wiedemann, *Chrysops aberrans* Philip, *C. univittatus* Macquart, *C. cincticornis* Walker, *Hybomitra lasiophthalma* (Macquart)), stable flies (*Stomoxys calcitrans* Linnaeus) (Diptera: Muscidae) and mosquitoes (*Aedes*) (Diptera: Culicidae) was investigated at various sites in Canada (Ontario, Alberta) and USA (Iowa, Florida, Louisiana). Traps made from selected fabrics, insect nettings and hand-dyed blue cotton were compared to the African design to provide practical recommendations for temperate environments. Comparisons of substituted materials showed that trap performance was optimal only when traps were made from appropriate fabrics in the colours produced by either copper phthalocyanine (phthalogen blue), or its sulphonated forms (turquoise). Fabrics dyed with other blue chromophores were not as effective (anthraquinone, disazo, formazan, indanthrone, triphenyldioxazine). An appropriate texture as well as an appropriate colour was critical for optimal performance. Smooth, shiny synthetic fabrics (polyester, nylon) and polyester blends reduced catches. Low catches occurred even for nominal phthalogen blue, but slightly-shiny, polyester fabrics in widespread use for tsetse. The most suitable retail fabric in place of phthalogen blue cotton was Sunbrella Pacific Blue acrylic awning/marine fabric. It was both attractive and durable, and had a matching colour-fast black. Nzi traps caught grossly similar numbers of biting flies as canopy, Vavoua, and Alsynite cylinder traps, but with differences in relative performance among species or locations.

**Keywords:** tabanids, stable fly, cluster fly, mosquitoes, Nzi trap, phthalogen blue

## Introduction

Biting flies vary from being a simple nuisance to being a serious economic and/or health burden to people and animals (Foil & Hogsette, 1994; Hall & Wall, 2004). When the use of insecticides for fly control is not preferred, or effective,

there are few devices that can be used on a 'Do-It-Yourself' basis. For stable flies (*Stomoxys calcitrans* Linnaeus) (Diptera: Muscidae), a sticky trap based on a fibreglass cylinder (Broce, 1988) is the only practical product (Olson Products, Medina, Ohio). Few traps catch many deer flies (French & Hagan, 1995), but sticky objects may be useful in reducing fly attacks (Cilek & Medrano, 2000; Mizell *et al.*, 2002). For other tabanids, a box-style canopy trap with a black decoy (Thompson, 1969) is marketed as the Horse-Pal<sup>®</sup> trap (Newman Enterprises, Omro, Wisconsin). A trap exploiting other visual decoys is the Epps trap (US Patent no. 5836104,

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Table 1. Details of the materials used in experiments.

Name	Colour	Material	Supplier
<b>Fabrics</b>			
Phthalogen Blue – standard	Phthalogen Blue	Cotton	Mount Kenya Textiles, Kenya
Phthalogen Blue	Phthalogen Blue	Cotton	Awassa Textiles, Ethiopia
Sunbrella Pacific Blue no. 6001	Phthalogen Blue	Acrylic	Glen Raven Mills, USA
SolarMax no. 6	Royal Blue	Shiny nylon	Dupont, USA
SolarMax no. 7	Peacock Blue	Shiny nylon	Dupont, USA
Sergeant no. 3006	Royal Blue	Polyester/cotton	J. Ennis Fabrics, Canada
Trigger poplin (Royal Box)	Royal Blue	Polyester/cotton	Galey and Lord, USA
Santiago	Phthalogen Blue	Polyester/cotton	Ets. Gonfreville, Ivory Coast
VF no.10 (1996)	Bright Blue	Polyester	Vestergaard Frandsen, Denmark
VF no. 11 (2001–2002)	Royal Blue	Satin-shiny Polyester	Vestergaard Frandsen, Denmark
TC new blue T-286 (2003)	~ Phthalogen Blue	Polyester	Vestergaard Frandsen, Denmark
Mixed yarn blue 286 (2003)	~ Phthalogen Blue	Polyester/viscose	Vestergaard Frandsen, Denmark
<b>Netting and other materials</b>			
VF no. 13 Netting – standard	White, 80 holes*	Polyester	Vestergaard Frandsen, Denmark
Insect Netting no. 5	Olive drab, 196 holes	Polyester	Army Navy, USA
Insect Netting no. 6	White, 526 holes	Polyester	Army Navy, USA
Insect Netting no. 8	Grey, 625 holes	Polyester	Army Navy, USA
Insect Screening	Charcoal, 225 holes	Fibreglass	Bay Mills, USA
Phifertex furniture fabric	White, 256 holes	Vinyl-coated polyester	Phifer Wire Products, USA
	Royal Blue, Black		
Tyvek sheeting	Translucent white	Polyolefin	Dupont, USA
<b>Trade Name</b>			
	Colour	Colour Index Code	Chromophore
<b>Blue dyes</b>			
IF3GM – standard	Phthlaogen Blue	Ingrain Blue 2:1	CuPc pigment developed in situ
Direct Blue 86	Light Greenish Blue	Direct Blue 86	Sulphonated CuPc
Procion Turquoise M-G	Greenish Blue	Reactive Blue 140	Sulphonated CuPc
Cibacron Turquoise M-G	Greenish Blue	No entry	Sulphonated CuPc
Procion Blue M-G	Royal Blue	Reactive Blue 163	Triphenodioxazine
Procion Blue H-EGN	Royal Blue	Reactive Blue 198	Triphenodioxazine
Procion Brilliant Blue M-R	Brilliant Blue	Reactive Blue 4	Anthraquinone
Remazol Brilliant Blue R	Brilliant Blue	Reactive Blue 19	Anthraquinone
Cibacron Blue F-R	Medium Blue	Reactive Blue 182	Formazan
Cibacron Navy F-G	Dark Navy Blue	Reactive Blue 204	Disazo
Vat Blue BC	Dark Blue	Vat Blue 6	Indanthrone derivative
Remazol Turquoise Blue G†	Greenish Blue	Reactive Blue 21	Sulphonated CuPc
LevaFix Brilliant Blue E-FFN†	Brilliant Blue	Reactive Blue 181	Anthraquinone

\*Mesh size in holes per square inch; †tested in Florida only with sticky panels.

Farnam Companies, Phoenix, Arizona). Painted wooden box traps are also used for tabanid control (Wall & Doane, 1980).

The Nzi trap, a 1-m triangular blue/black cloth trap with two blue wings for tsetse and biting flies, was recently described (Mihok, 2002). It was the product of research on attractive devices for biting flies in Africa, guided by studies on tsetse (Green, 1994). This simple and economical trap is suitable for consumer use. It typically catches more biting flies than other traps, which are often optimal for just a few groups. Except for large interception traps such as the Malaise, good trap performance for all species is difficult to achieve (Gibson & Torr, 1999). The Nzi trap was designed for equitable efficiency for tsetse, stable flies and tabanids. It has been tested extensively in the tropics, but few studies have been conducted elsewhere. Experiments were therefore initiated to investigate the suitability of the trap for temperate areas. Detailed studies were done in Ontario, Canada with exploratory work at other locations in North America. The objective was to test trap performance in relevant contexts to facilitate straightforward use by both researchers and the public.

## Materials and methods

### Standard trap

New or nearly-new Nzi traps were used as controls. Standard traps (STD) were made from cotton drill dyed by Mount Kenya Textiles (Nanyuki, Kenya) with sulphur black, and phthalogen blue IF3GM (fabric no. 1b in Mihok, 2002). This process develops copper phthalocyanine (CuPc, Pigment Blue 15) in cotton (Vollmann, 1971). Its spectral reflectance was identical to historical fabrics (Green, 1989), and matched a modern sample of type IF3GM from Dystar Textilfarben GmbH, Frankfurt, Germany. The rest of the trap was made from Vestergaard Frandsen Group (VF, Kolding, Denmark) no. 13 white polyester netting (fluorescent, fabric no. 25 in Mihok, 2002). Details of fabrics and other materials for all experiments are summarized in table 1.

### Study areas

In Canada, unbaited STD traps were set first at Lethbridge, Alberta (49°42'N, 112°47'W) from August to November 1999 in applied settings near cattle or horses. Monitoring

continued in a rural residential area at Russell, Ontario (45°15'N, 75°21'W) from May to November 2001; experiments commenced in 2002. In the USA, complimentary experiments were conducted at the USDA-ARS-CMAVE in Gainesville, Florida (29°41'N, 82°16'W), at a dairy farm in Ames, Iowa (41°59'N, 93°37'W); and at an agricultural centre (30°15'N, 91°06'W), and a natural area in Louisiana (30°39'N, 92°00'W).

### Experimental designs

Experiments were nearly all Latin square designs (Perry *et al.*, 1980) with a focus on Nzi traps made from different fabrics, netting, and dyed cloth, along with selected comparisons to other traps. Reflectance from 370 to 830 nm was measured for all materials with a USB 2000 Fibre Optic Spectrophotometer (Ocean Optics Inc., Dunedin, Florida) using a simple apparatus (Cilek, 2003). Readings were standardized for 100% reflectance with barium sulphate. Raw catch and spectral reflectance data, and samples of most fabrics, are available on request.

### Ontario

At Russell, experiments were mostly 4 × 4 to 6 × 6 Latin square designs with 3–4 replicates, providing a median sample size of 17 per treatment. Experiments were conducted in a turfgrass area on a 0.5-ha residential property, with scattered trees and adjacent woodlots. Typically, traps were set at ground level, 7–25 m apart, facing west, on a north-south transect (crosswind), and baited with a wax formulation of 1-octen-3-ol (octenol, Biosensory Inc., Willimantic, Connecticut). Practical aspects for the operation of Nzi traps are provided at nzitrap.com.

### Fabrics and netting

Synthetic fabrics in similar blues were tested as replacements for phthalogen blue cotton across nine experiments (table 1). Matching black fabrics from the same source were used in most cases; otherwise black cotton twill was substituted. Four retail fabrics were tested along with four fabrics designed for tsetse. The Santiago fabric was identical to the historical material (Laveissière *et al.*, 1987a). The royal blue VF no. 11 was a new, satin-shiny version of fabric no. 23 referred to as 'matt royal blue, pongee 1' in Mihok (2002). The VF 286 'new blue' fabrics were new colours of VF no. 10 ('matt pure blue, pongee 2', fabric no. 24 in Mihok, 2002). Five mesh products in neutral tones (white, grey, charcoal, olive drab) were tested in place of VF no. 13 netting across six experiments (table 1). White Tyvek (translucent sheeting) and 'royal' blue Phifertex mesh (~ phthalogen) were tested as substitutes for back netting only. Lastly, an all-Phifertex mesh trap (royal blue, black, white) was tested.

### Dyes

To test colour independently of appearance, material differences were eliminated by hand-dyeing cotton (table 1) in a representative series of ten blues (Shore, 1995). Except for Direct Blue 86 (very light) and Cibacron Navy F-G (very dark), cloth was dyed to about the same shade as the phthalogen blue standard (5% by fabric weight). To examine the importance of colour saturation, a faded phthalogen blue trap, and a light shade of Procion Turquoise M-G

matching Direct Blue 86, were also tested. Dyed fabrics were compared in four experiments in 2004; in each test only the phthalogen blue cotton was replaced with an alternative blue.

### Other biting fly traps

A few conventional traps were tested in four experiments at peak numbers. For tabanids, a canopy trap (Hribar *et al.*, 1991), set 60 cm high, was tested in summer 2004. For stable flies, a Vavoua trap (Laveissière & Grébaud, 1990), set 15 cm high, was tested in September 2003. The retail version of the Alsynite cylinder trap (Broce, 1988) was tested in September 2004. This is a sticky trap that employs a transparent sleeve with a dry adhesive attached to a small translucent fibreglass cylinder (30 cm high, 18 cm wide). To test the uniqueness of the optical properties of fibreglass (Zacks & Loew, 1989), a plain Alsynite cylinder trap, and one with Procion Turquoise M-G cloth completely covering the fibreglass on the outside were compared. A sticky sleeve with no cylinder was set nearby on a thin cylindrical wire frame to monitor any innate attractiveness of the adhesive (Brach & Trimble, 1985).

### Targets and fly behaviour

Four beach balls were tested as hanging decoys in the body of STD traps during the peak of the tabanid season. A 45-cm diameter (inflated) striped ball (Cilek, 2002) was used as is, or spray-painted glossy black or blue, along with a larger striped ball (55 cm). Alsynite cylinders were also tested as trap accessories in three experiments during peak stable fly numbers; in one of these experiments traps were set facing east or west as a separate experimental factor. Cylinders with or without turquoise cloth were placed inside STD traps in several positions. In the last experiment, a cylinder with a sticky sleeve was used to monitor activity just inside the front entrance. Stable flies and cluster flies (*Pollenia rudis* Fabricius, Calliphoridae) were at high numbers at this time. Sticky sleeves were therefore attached to the front blue surfaces of a nearby Nzi trap for five days to monitor fly activity. Two experiments at eight sites were ongoing at this time, allowing for simultaneous comparisons of sticky sheet catches in six contexts: on three blue trap surfaces, on an Alsynite cylinder set on its own or set inside a Nzi trap, and on a turquoise cloth cylinder set on its own.

### Florida

Several exploratory trials were conducted to guide summer work in Ontario. These trials used 100–500, 4- to 9-day-old, mixed-sex, laboratory-reared stable flies (Hogsette, 1992) released daily into screened, outdoor enclosures (7 × 8 × 3 m high or 10 × 20 × 7 m high). Tests were randomized trials or Latin square designs run for 4–12 days with four devices compared simultaneously in one enclosure. In two initial trials, sticky sheet catches on 30 × 30 cm panels with phthalogen blue cloth were compared to those with Sunbrella Pacific Blue fabric, and two generic fabrics (indigo denim cotton, medium blue plain cotton). In two further trials, phthalogen blue cotton, Sunbrella, and shades of 'imitation' phthalogen blue (custom-dyed mixtures of RB 21 + 181, table 1) were compared. In a final series with traps, catches were compared for a new and a weathered STD trap,

a new Sunbrella trap, and a new VF no. 11 royal blue polyester trap. Traps were not baited for 6 days; for the last 4 days they were baited with acetone and a 16:1:8 mixture of phenols and octenol (4-methylphenol:3-propylphenol:1-octen-3-ol, Torr *et al.*, 1997).

#### Iowa and Louisiana

Nzi traps in matching blue and black fabrics (cotton, polyester, or nylon), unbaited, and all with white polyester netting, were compared in 3-times replicated 3×3 Latin squares at a dairy farm in Ames, Iowa in July 1996. The blue nylon was similar to the SolarMax royal blue (table 1). The other traps were a STD trap from Ethiopian phthalogen blue cotton (fabric no. 26 in Mihok, 2002), and a trap in VF no. 10 bright blue polyester (previously tested in experiment 23 of Mihok, 2002). A similar experiment was conducted in June 1996 along a grassy road verge in bottomland forest at the Thistlewaite Wildlife Management Area, Louisiana, with a canopy trap (Catts, 1970) replacing the nylon Nzi trap. It had a black beach ball and was made with a light brown saran screen (ultraviolet absorbing). Traps were baited with an 8:1:4 blend of phenols and octenol (Torr *et al.*, 1997). Lastly, a VF no. 11 royal blue polyester Nzi trap was compared to a Trigger poplin trap (table 1), and an Alsynite cylinder trap, at the Louisiana State University Agricultural Center, St Gabriel Research Station, Louisiana in spring 2003. Stable flies only were counted. Traps were unbaited and were set in a 2-times replicated 3×3 Latin square.

#### Statistical analyses

Catches  $x$  were transformed as  $y = \log(x+1)$  for ANOVA. The outcome of interest was an *a priori* significant change in catch relative to the control, typically a STD trap. Proportionate changes in catch were summarized as the response ratio ( $R$ ) of each treatment relative to the control (Hedges *et al.*, 1999), i.e. the ratio of the detransformed mean catch in the test trap to that in the standard. This measure of effect size in meta-analysis is the equivalent of the 'catch index' or 'index of increase' in entomological publications (Dransfield & Brightwell, 1992). Results are presented in terms of  $R$  (lower 95% CI–upper 95% CI), with geometric mean catches for the control ( $GM_T = R \cdot GM_C$ ) also provided for interpretation. Duplicate entries in figures with different sample sizes reflect data from separate experiments.

## Results

### Canada

During monitoring with an unbaited trap at Lethbridge near livestock, 17,641 stable flies (66% male) were captured, with a maximum of 1618 stable flies per trap per day (mean ~250 per day). In 1688 trap-days of effort at Russell, the catch was 6708 tabanids (60% *Tabanus*, 35% *Chrysops*, 5% *Hybomitra*), and 6024 stable flies (70% males). Common tabanids were *T. similis* Macquart (30%), *T. quinquevittatus* Wiedemann (29%), *Chrysops aberrans* Philip (16%), *C. univittatus* Macquart (8%), *C. cincticornis* Walker (8%), and *Hybomitra lasiophthalma* (Macquart) (5%). Mosquitoes were also captured ( $n = 3542$ , 80% *Aedes*) with data obtained for 45 variations on Nzi traps. As mosquito catches were not

significantly improved relative to a STD trap, these results are not mentioned further.

### Fabrics and netting

Sunbrella was the only synthetic fabric that provided similar catches relative to cotton. Altogether, six variations on Sunbrella were tested across six experiments, along with selected substitutions of another outdoor fabric, Phifertex mesh (fig. 1). For stable flies, Sunbrella and/or Phifertex substitutions resulted in catches equal to or significantly better than those in a STD trap. The only trap that was not effective for stable flies was one made entirely out of Phifertex mesh ( $R = 0.31$ ,  $GM_C = 3.9$ ). In contrast, an all-Phifertex trap caught many tabanids, with only a slightly-reduced catch of *Chrysops*. Overall, catches of Tabanidae with Sunbrella and/or Phifertex substitutions were identical to those in a STD trap (mean  $R = 0.98$ ), but with different patterns among genera (fig. 1b). In the experiment with the highest catch, a Sunbrella trap caught more *C. cincticornis* ( $R = 1.38$  (1.05–1.81),  $GM_C = 1.5$ ), equal numbers of *C. aberrans* ( $R = 1.06$  (0.82–1.36),  $GM_C = 2.3$ ), fewer *T. similis* ( $R = 0.63$  (0.47–0.85),  $GM_C = 10.3$ ), and fewer *T. quinquevittatus* ( $R = 0.60$  (0.45–0.80),  $GM_C = 9.7$ ) than a STD trap.

Four types of mesh other than Phifertex were compared to standard VF no. 13 netting across four experiments (data not shown). Equal catches of all biting flies were obtained only with fine white netting similar to the standard (not fluorescent, 526 vs. 80 holes, table 1). However, catches with other neutral tones of netting were no worse than 50–70% of those with white netting, with confidence intervals approaching  $R = 1.0$ . Translucent white Tyvek was tested for stable flies only, and only at the back of the trap, with poor results ( $R = 0.28$  (0.20–0.39),  $GM_C = 3.9$ ). Catches with synthetic fabrics other than Sunbrella were lower in all comparisons across six experiments (fig. 2). Particularly low catches were obtained with VF polyester fabrics designed for tsetse. The 286 'phthalogen blue' fabrics were tested at low stable fly numbers ( $GM_C = 1.5$ ), and at high tabanid numbers ( $GM_C = 25.3$ ). As a typical example,  $R$  was only 0.16 (polyester) and 0.21 (mixed yarn) for *T. quinquevittatus*. Two experiments were conducted with VF no. 11 royal blue polyesters at high stable fly numbers ( $GM_C = 10.7$ , 16.3). Traps in slightly different fabrics purchased in 2001 and 2002 caught very few stable flies ( $R = 0.28$ , 0.07 respectively). As the season ended, an attempt was made to understand these poor results by comparing a modified VF trap to a STD trap in a well-replicated test ( $n = 20$ ). The modification was to cover the VF blue fabric with phthalogen blue cotton. This manipulation was only partially effective in terms of improving catches ( $R = 0.36$ ,  $GM_C = 6.8$ ).

### Dyes

Only traps in greenish-blue colours produced by sulphonation of the CuPc chromophore provided equitable catches of all biting flies relative to the brilliant blue produced by CuPc itself (fig. 3). Response ratios for other dyes (table 1) were nearly all significantly lower (data not shown), averaging 0.3 for tabanids (range 0.2–0.5, all  $P < 0.05$  relative to the STD trap), and 0.6 for stable flies (range 0.4–0.9). Results for the four contrasting dyes tested at the highest stable fly numbers had an average  $R$  of 0.4 (all  $P < 0.05$ ). For tabanids, even a good colour match to the human eye (colour difference index CMC (2:1)  $\Delta E = 4$ , relative to typical

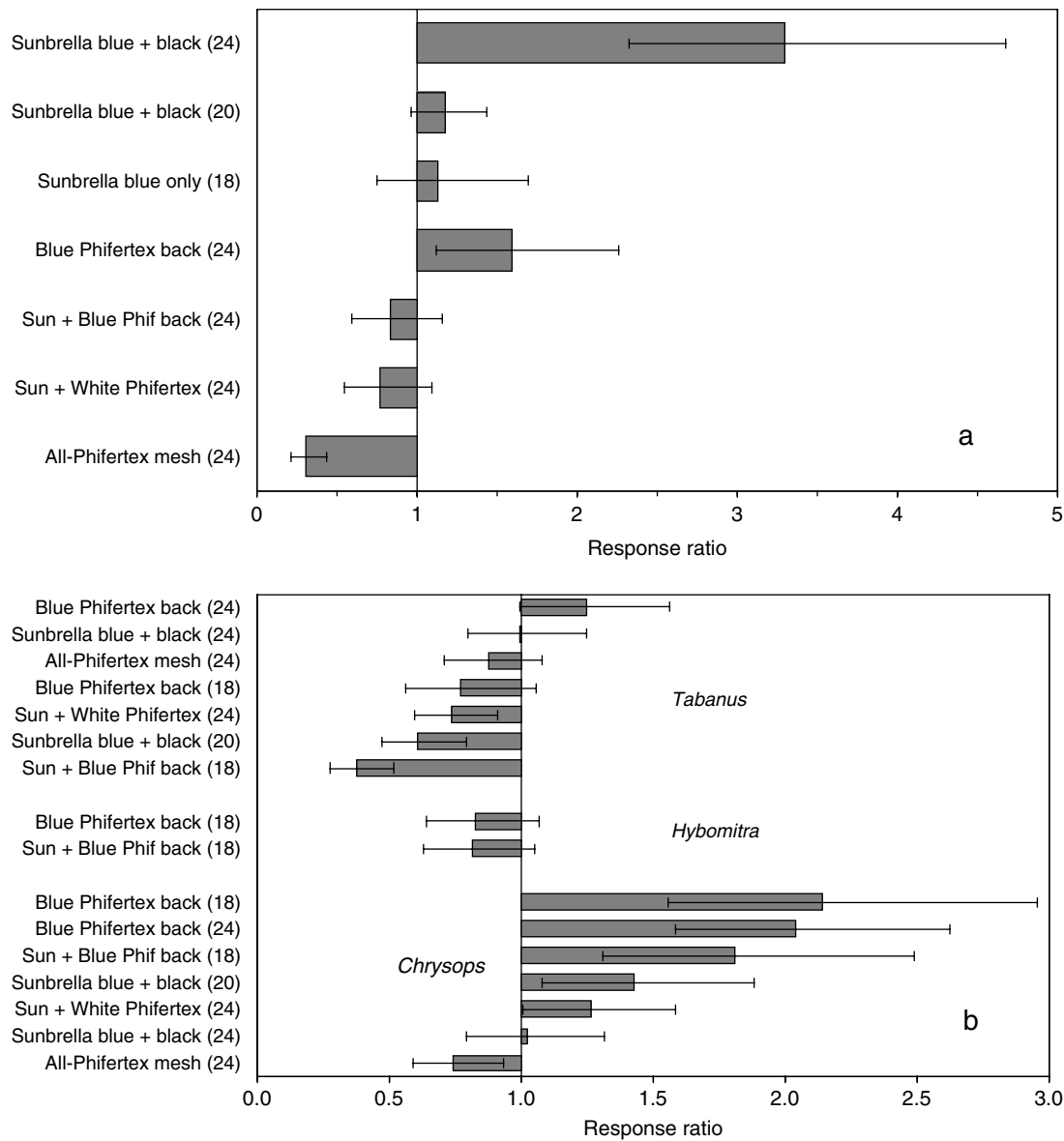


Fig. 1. Response ratios ( $R$ ) for Nzi traps with various combinations of Sunbrella acrylic fabric and/or Phifertex mesh relative to a STD cotton trap. Note the different scales for stable flies (a) and tabanids (b). Six experiments are represented; sample sizes are in parentheses. Duplicate entries represent the same trap in separate experiments. Sun is an abbreviation for Sunbrella blue + black; Phif is an abbreviation for Phifertex.

industry objectives of just a few units, Nassau, 1998), catches with the triphenyldioxazine blue of Procion Blue H-EGN were very low ( $R=0.21$ ,  $(0.15-0.21)$ ,  $GM_c=9.5$ ).

For CuPc-based dyes (fig. 3), stable flies were the least sensitive to colour saturation, with a just significantly lower catch with the very light shade produced by Direct Blue 86. In contrast, catches of tabanids were lower with all light shades, including a faded phthalogen blue trap. This result was striking when a dark shade of Procion Turquoise M-G caught eight times as many tabanids ( $R=2.35$ ) as Direct Blue 86 ( $R=0.30$ ) in a simultaneous test. A higher catch of tabanids (80% *T. quinquevittatus* + *T. similis*) with Procion Turquoise M-G was documented only as tabanid numbers

waned. When a similar colour (Cibacron Turquoise F-G) was tested at lower numbers, there was no increase in catch for a fauna dominated by *Chrysops* (fig. 3).

#### Other biting fly traps

The Vavoua trap caught equal numbers of stable flies relative to a STD trap ( $R=1.14$ ,  $(0.82-1.60)$ ,  $GM_c=3.9$ ). The canopy trap caught very few tabanids, five times less than a STD trap ( $R=0.21$ ,  $(0.14-0.31)$ ,  $GM_c=14.8$ ), with low catches of both *Tabanus* and *Chrysops*. Mid-way through this trial, a black beach ball was suspended under the trap; the catch remained low. An Alsynite cylinder trap caught more

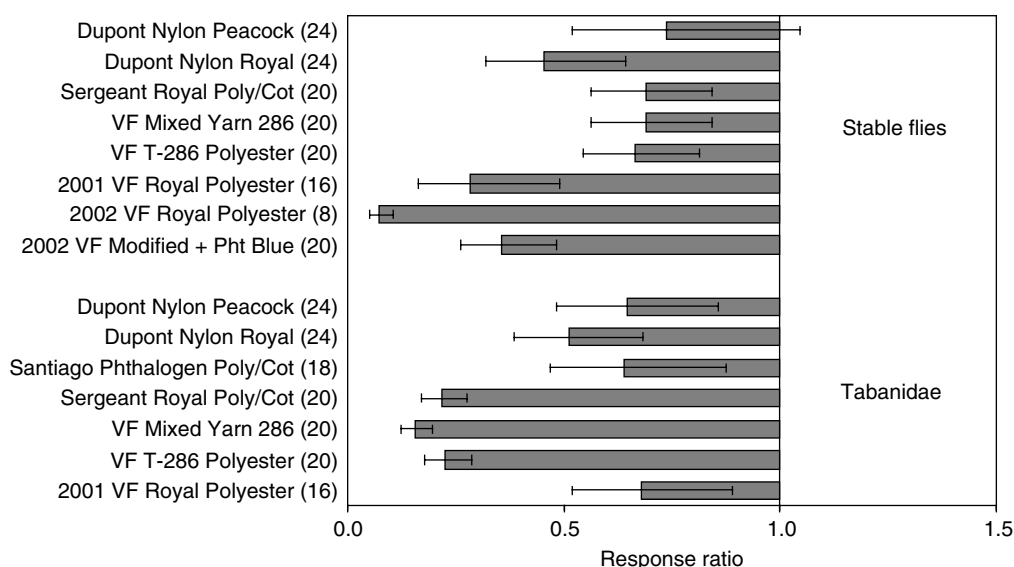


Fig. 2. Response ratios ( $R$ ) of stable flies and tabanids for Nzi traps made out of synthetic fabrics or blends relative to a STD cotton trap. Six experiments are represented; sample sizes are in parentheses. The '2002 VF Modified + Pht Blue' trap is the same trap as '2002 VF Royal Polyester' trap but with phthalogen blue cotton drill covering the royal blue polyester.

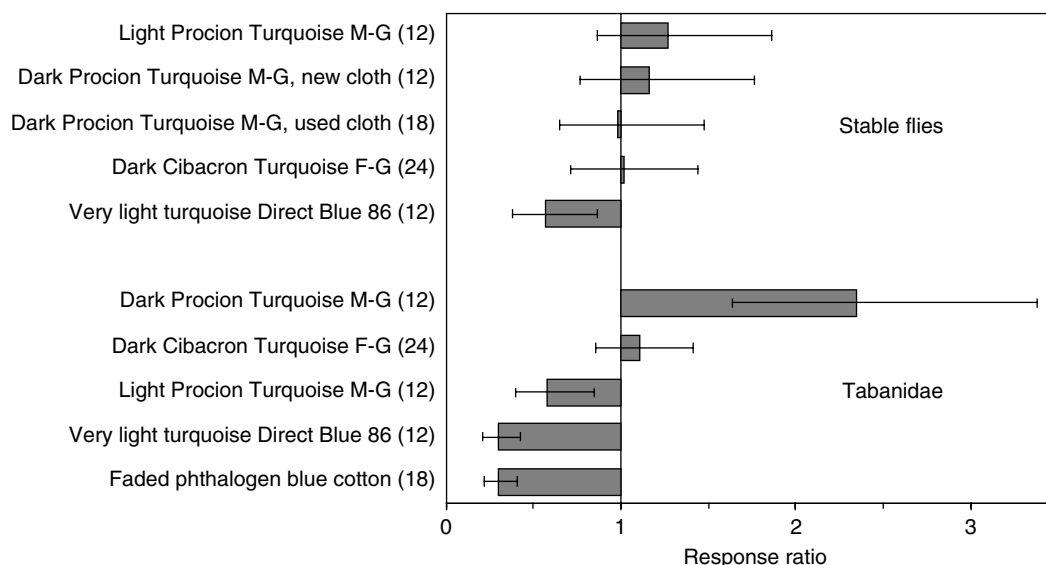


Fig. 3. Response ratios ( $R$ ) of stable flies and tabanids for phthalogen blue and greenish-blue (turquoise) Nzi traps based on copper phthalocyanine chromophores (CuPc) relative to a STD cotton trap. Five experiments are represented; sample sizes are in parentheses.

stable flies ( $R = 2.72$ ,  $1.81\text{--}4.09$ ),  $GM_c = 4.5$ ) than a STD trap. The experiment comparing a plain cylinder to one covered in turquoise cloth was done at high numbers of both stable flies ( $GM = 24.2$ ) and cluster flies ( $GM = 19.5$ ). Catches with a cylinder covered in opaque turquoise cloth were equivalent to those on a plain cylinder without cloth (stable flies:  $R = 1.18$ ,  $0.81\text{--}1.72$ , cluster flies:  $R = 1.07$ ,  $0.65\text{--}1.76$ ). A sticky sleeve set alone caught no flies.

#### Targets and fly behaviour

When added to STD traps, blue, black or striped balls did not affect the catch of tabanids ( $R$  from 0.77 to 0.92,

$GM_c = 6.0$ ), or stable flies ( $R$  from 0.88 to 1.12,  $GM_c = 1.8$ ). When placed inside the body of STD traps, Alsynite cylinders, with or without turquoise cloth attached, also did not affect the catch of stable flies or cluster flies ( $R$  from 0.72 to 1.08). Many stable flies were caught on a sticky cylinder set just inside the entrance of a trap ( $GM = 28.4$ ); resulting in a slightly lower trap catch ( $R = 0.51$ ,  $0.31\text{--}0.84$ ). In contrast, few cluster flies ( $GM = 3.6$ ) were caught, with no effect on the trap catch ( $R = 0.99$ ,  $0.61\text{--}1.60$ ). When trap orientation was tested, catches of stable flies were nearly doubled when traps faced west ( $R = 1.80$  ( $1.12\text{--}2.91$ ),  $GM_c = 3.7$  for east). The opposite was true for cluster flies ( $R = 0.58$  ( $0.43\text{--}0.77$ ),  $GM_c = 3.3$  for east). When sticky sheets

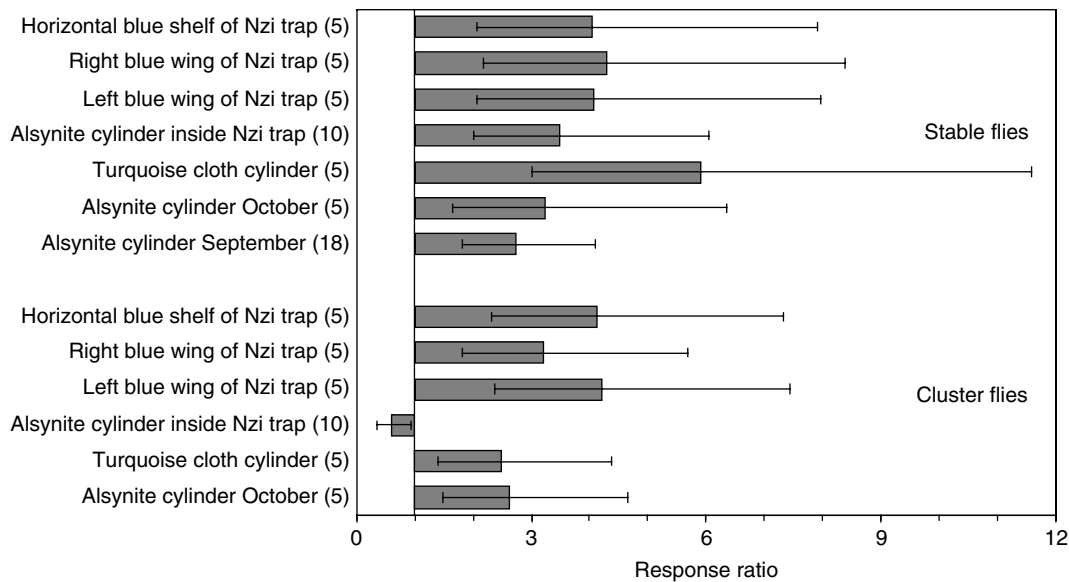


Fig. 4. Response ratios ( $R$ ) of stable flies and cluster flies for catches on transparent sticky sheets attached to various objects relative to catches in STD cotton traps ( $GM_c$  stable flies = 8.2,  $GM_c$  cluster flies = 6.0,  $n = 10$ ). Except for an Alsynite cylinder tested in September, data were collected simultaneously over 5 days in October, 2004.

were used simultaneously on trap surfaces, and on plain or turquoise cloth cylinders set on their own or inside traps, catches of stable flies were equivalent in all contexts ( $GM_{sticky} = 32.6$ ). The same was true for cluster flies ( $GM_{sticky} = 12.1$ ), with one exception (fig. 4). Catches on a sticky sheet in all contexts for both species were mostly 2–4 times higher than those in a STD trap. Stable fly sex ratios were slightly male-biased (57% male,  $n = 1356$ ), with no significant difference among contexts ( $\chi^2 = 10.0$ ,  $df = 6$ ,  $P = 0.12$ ).

On average, 110.6 stable flies per day were caught on the sticky sheets attached to the front of a Nzi trap, relative to 5.2 caught in the trap itself, and relative to 9.6 caught in the control (STD traps). The totals for cluster flies were 75.6, 1.0, and 5.2. If the sticky sheets intercepted all flies at the trap, these data would suggest an efficiency of 8.3% for stable flies ( $9.6/(110.6 + 5.2)$ ), and 6.8% for cluster flies. These low estimates were obtained on cool days (mean daily maximum of 20.6°C) when flies were active only for a few hours late in the afternoon.

#### USA

In Florida in each sticky panel test, more stable flies were attracted to phthalogen blue cotton than to any other fabric, with 131–185 flies caught in total per panel, and with 38–63% recovery of released flies. Catches with Sunbrella and imitation phthalogen blue were only slightly lower, and not significantly different (data not shown). Catches were significantly lower for other generic blues (30–44% of those for phthalogen blue). The efficiency of Nzi traps operated inside an enclosure was poor, with only 6% of the flies recovered. The two phthalogen blue traps performed best, catching twice as many flies (97, 104) as the Sunbrella trap (42), and three times as many as the VF no. 11 royal blue polyester trap (29).

In Iowa, 16,472 stable flies (63% male) were captured with a maximum of 1497 per trap per day. The VF no. 10 bright blue polyester trap caught similar numbers of stable flies as the standard phthalogen blue cotton trap (males:  $R = 1.00$ , (0.52–1.93),  $GM_c = 70.0$ , females:  $R = 0.76$ , (0.42–1.39),  $GM_c = 46.1$ ). The shiny, royal blue nylon trap performed poorly (males:  $R = 0.26$ , (0.14–0.51), females:  $R = 0.27$ , (0.15–0.49)). In Louisiana, 4927 tabanids (66% *Tabanus fuscicostatus* Hine, 17% *T. lineola* complex, 9% *Leucotabanus annulatus* (Say), 3% *T. limbatinervis* Macquart) were captured with a maximum catch in a Nzi trap of 121 per day ( $GM_c = 45$  tabanids). With the exception of a lower catch for the *T. lineola* complex ( $R = 0.44$ , (0.34–0.57)), the VF no. 10 polyester trap caught similar numbers of each taxon as the cotton trap ( $R = 0.87$  to 1.24, all  $P > 0.05$ ). The canopy trap caught more *T. fuscicostatus* ( $R = 2.60$ , (2.13–3.18)), fewer *L. annulatus* ( $R = 0.56$ , (0.41–0.77)), and similar numbers of *T. lineola* complex ( $R = 0.97$ ) and *T. limbatinervis* ( $R = 0.87$ ) as the cotton Nzi trap. At the agricultural centre in Louisiana, the Trigger poplin royal blue Nzi trap caught the most stable flies ( $GM_c = 56.3$ ). Relative to this trap, catches were similar in the Alsynite cylinder trap ( $R = 0.75$  (0.47–1.19)), and significantly lower in the VF no. 11 royal blue polyester Nzi trap ( $R = 0.55$  (0.35–0.88)).

#### Discussion

Nzi traps captured a wide variety of biting flies in North America as in Africa (Mihok, 2002), with reasonable catches relative to canopy traps for tabanids, and relative to Alsynite or Vavoua traps for stable flies. At a residential site in Ontario where biting flies were not conspicuous, up to 67 tabanids, 71 stable flies, 46 deer flies, and 121 mosquitoes were caught per day. When traps were set by livestock in Alberta and Iowa (this paper), and in Florida (unpublished data), they caught hundreds to well over a thousand

stable flies per day. Similar catches of other *Stomoxys* spp. are typical in Africa (Abeeluck *et al.*, 2001; Mihok, 2002; Ndegwa & Ogodo, 2002). For other Stomoxyinae (Mihok *et al.*, 1996), and for ceratopogonids and simuliids, trap performance has yet to be documented. Along with the test conducted here in Louisiana, several studies have already demonstrated efficacy for diverse tabanids, e.g. in Kenya (Mihok, 2002), the Ivory Coast (Acapovi *et al.*, 2002), Chad (Doutoum *et al.*, 2002), Mali (IAEA, 2003), Brazil (Koller *et al.*, 2003), and Burkina Faso (Desquesnes & Dia, 2004). Although species and locality differences need to be considered for optimal trap selection, the Nzi trap met expectations as a useful generic sampling device for biting flies in North America.

To test whether performance could be improved with simple accessories, beach balls or Alsynite cylinders were added to Nzi traps. Catches were not affected by the presence of these potentially attractive objects. For tabanids, decoys may be useful only when set alone, or when used against a background of transparent features (Bracken & Thorsteinson, 1965; Schreck *et al.*, 1993). For stable flies, sticky catches demonstrated considerable fly activity on an Alsynite cylinder decoy placed just inside the trap entrance, but this activity did not translate into an improved catch. Close-range behaviour was difficult to alter, as in a similar attempt to improve Nzi traps by using a push-pull strategy of attractive and unattractive fabrics (Mihok, 2002).

Features of the immediate environment are known to have major effects on the behaviour of tsetse (Vale, 1998), but similar insights have yet to be gained for biting flies. With a single entrance, Nzi trap efficiency could be dependent on orientation to the sun, wind or nearby objects. Tests in Ontario were therefore standardized by setting traps cross-wind in a large, open area, facing west. This practice was adopted after documenting high activity using a transparent sticky enclosure on trap surfaces facing the afternoon sun, as well as higher catches of tabanids in traps facing west (unpublished data). Given upwind flight in odour plumes (Gibson & Torr, 1999), wind effects on landing behaviour (Broce *et al.*, 1991; Cilek, 2003), and westerly winds, an eastern trap orientation was initially chosen. However, once behaviour was documented, it was clear that flies investigated sunny sides of a trap, regardless of other factors. Due to the fast flight of tabanids, close-range behaviour could not be observed. For stable flies, the behaviour resulting in higher catches in west-facing traps was self-evident at high numbers; e.g. in late summer (Lysyk, 1993). Males especially used the front of traps as 'waiting stations' on warm afternoons (Buschman & Patterson, 1981). This behaviour was also seen in Florida, and was perhaps reflected in male-biased catches (63% Iowa, 66% Alberta, 70% Ontario).

Considerable effort was placed on finding a common retail fabric that could substitute for phthalogen blue cotton, now a historical fabric (Vollmann, 1971) with limited production. In tests against other colours in Africa, phthalogen blue cotton has been the ideal material for attracting both tsetse (Green, 1988), and biting flies (Mihok, 2002). As a result of global market forces (Park & Shore, 1999), phthalogen blue cotton is now essentially unavailable outside of the developing world. Fortunately, a widely-distributed fabric, Sunbrella in Pacific Blue, proved to be an excellent substitute. Pacific Blue is a good colour match to the eye (CMC (2:1)  $\Delta E = 3$ ), with excellent spectral correspondence in the critical blue/ultraviolet range. There are

minor differences in appearance (e.g. fluorocarbon finish), but these seem to have only a minimal impact on catches (e.g. *Tabanus* in fig. 1b). Pacific Blue is likely produced with CuPc through solution dyeing (Herbst & Hunger, 2004). We therefore recommend this fabric as an alternative standard for traps, whenever phthalogen blue IF3GM cotton, cannot be obtained.

For other fabrics, substitutions of similar colours and/or materials for phthalogen blue cotton often resulted in reduced catches. Subtle details of appearance such as texture and gloss were just as important for performance as more obvious features of lightness (depth of shade), chroma and hue (colour) (Gilbert *et al.*, 2005). Particularly low catches of biting flies were obtained with several VF polyesters; these or similar fabrics have been used in large-scale control operations for tsetse (Abel *et al.*, 2004). Numerous VF fabrics have been marketed since 1995, but their characteristics and performance remain largely undocumented (Mihok, 2002). In contrast, the original adoption of phthalogen blue cotton for tsetse (Ryan & Molyneux, 1980) had a strong foundation in experimental studies (Green, 1994). Later, once synthetic fabrics were shown to retain insecticides well (Laveissière *et al.*, 1987b), some researchers shifted to other fabrics. Market forces continue to play a major role in how fabrics are chosen (Okello-Onen *et al.*, 2004).

Fly behaviour towards subtle visual cues in blue fabrics was tested here for the first time through targeted comparisons of very similar colours in contrasting materials, and through careful tests of hand-dyed blue cotton, holding materials constant. Mixtures of paints have been used for similar, but much broader colour science objectives by other researchers (Allan & Stoffolano, 1986; Green & Flint, 1986). Past studies have demonstrated that tsetse and biting flies are broadly attracted to blue and/or dark objects (black or red), as perceived by the dipteran eye (Gibson & Torr, 1999). The ecological correlates of this phenomenon remain obscure (Steverding & Troscianki, 2004). Here we have shown that optimal catches of all types of biting flies can only be achieved with non-shiny fabrics, i.e. a necessary condition. As a sufficient condition, fabrics should also closely mimic the exact spectral profiles of CuPc or its sulphonated derivatives. We found that other blues produced by indanthrone, formazan, anthraquinone, triphenyldioxazine, and disazo chromophores were not optimal, even with good colour matches in terms of human vision. The uniqueness of 'phthalogen blue' to the dipteran eye has been noted in other empirical studies (Green & Flint, 1986; Green, 1988), but interpretation has been confounded by testing fabrics that differ simultaneously in appearance, as well as in colour (Mihok, 2002).

Excellent catches of both tabanids and stable flies were obtained here with two of the many sulphonated CuPc reactive dyes that can produce deep shades of greenish blue (SDC & AATCC, 1971). This shade of turquoise was particularly effective for *Tabanus*, possibly as a function of enhanced green sensitivity in the retina (Allan *et al.*, 1991). This observation is worthy of further investigation as deep turquoise fabrics have not often been tested. Detailed previous tests have been done with fabrics in a lighter turquoise similar to Direct Blue 86 (Ndegwa & Mihok, 1999; Mihok, 2002), which was not effective here. Turquoise-dyed cotton could provide a practical alternative for phthalogen blue cotton, given simple dyeing processes, useful auxiliaries, and high light fastness (Batchelor *et al.*, 2003).



In tests of alternatives to white netting, use of convenient retail products in neutral tones had only minor effects on catches. Lower catches were likely related to reduced light transmittance through the cone, which is essential for upward movement of flies in similar traps (Brightwell *et al.*, 1991; Vale, 1998). In contrast, use of blue mesh at only the back of the trap repeatedly doubled catches of deer flies. An eminently-practical trap for windy environments (all-Phifertex mesh) also caught many tabanids, but few stable flies. These contrasting patterns are similar to results for different tsetse species in epsilon traps equipped with blue 'windows' (Vale, 1998). During development of the Nzi trap (Mihok, 2002), equitable catches of all kinds of tsetse and biting flies were achieved only after testing numerous configurations of transparent panels and shelves. Only simple materials were tested, and hence further tests of products with special optical properties, e.g. horticultural mesh (Bell & Baker, 2000), or thermoplastics (Costa *et al.*, 2002) may help to improve trap efficiency. Ultraviolet cut-offs and other optical properties of visibly-transparent materials (Zacks & Loew, 1989) can greatly affect catches of biting flies (Agee & Patterson, 1983; Hribar & Foil, 1994). This was shown here with the low catch of stable flies with white Tyvek (high ultraviolet reflectance) relative to other bright white materials without this feature (fine white netting, white Phifertex mesh). Given the often dramatic effects of ultraviolet features of objects on fly behaviour (Gibson & Torr, 1999), tests of special types of mesh or sheeting (clear vinyl, acrylic, polycarbonate) with high blue, but low ultraviolet transmittance, are a fruitful area for further research.

### Acknowledgements

The authors thank John Shore for practical insights on dyeing fabrics, Torben Vestergaard Frandsen for supplying VF fabrics, Marc Desquesnes for providing Santiago fabric, Mandi Falkner for technical assistance, and G. White and J. Hogsette for comments on the draft manuscript.

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(Accepted 3 May 2006)  
CAB International, 2006

